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(54) Title: METHOD OF TREATING OTITIS MEDIA WITH URIDINE TRIPHOSPHATES AND RELATED COMPOUNDS

(57) Abstract

A method of promoting drainage of congested middle ear fluid in a subject in need of such treatment is disclosed. The method comprises administering to the middle ear of the subject a uridine triphosphate such as uridine 5'-triphosphate (UTP), an analog of UTP, or any other analog, in an amount effective to promote drainage of congested middle ear fluid by hydrating mucous secretions in the middle ear or by stimulating ciliary beat frequency in the middle ear or eustachian tube. The method is useful for treating patients afflicted with otitis media and other middle ear diseases, otitis externa, and inner ear diseases including Ménière's Disease. Pharmaceutical formulations and methods of making the same are also disclosed. Methods of administering the same would include any liquid suspension (including nasal drops or spray), oral, inhaled by nebulization, topical, injected or suppository form.

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METHOD OF TREATING OTITIS MEDIA WITH URIDINE TRIPHOSPHATES AND RELATED COMPOUNDS

(Case No. 96,027-A)

INTRODUCTION

Technical Field

This invention relates to a method of removing or preventing the accumulation of retained mucous secretions from the middle ear of a patient by administering certain uridine, adenosine, or cytidine triphosphates.

Background of the Invention

Otitis media (OM) is a viral or bacterial infection of the middle ear primarily, but not exclusively, afflicting children under three years of age. It is characterized by the presence of congested fluid in the middle ear and is usually precipitated by an infection in the respiratory tract which spreads into the middle ear via the nasopharnyx and eustachian tube. The incidence of OM is increasing--annual physician's office visits for OM have increased 150% from 1975 through 1990 (L. McCraig and J. Hughes, *JAMA* 273(3), 214-19 (1995)). This is most likely due to increased use of large-group day care facilities, where children are exposed to more respiratory pathogens. Approximately 25-40 million office visits are made each year for diagnosis and treatment of OM, and by age three, approximately 75% of children will have had at least one episode of acute OM (with the maximum incidence in children 6-24 months of age) (J. Klein, *Clin Infect Dis* 19, 823-33 (1994)). Anatomically, the eustachian tubes in infants are shorter, wider, and lie more horizontally than in older children and adults, facilitating the

spread of pathogens from the nasopharnyx to the middle ear (L. Schwartz and R. Brown, *Arch Intern Med* 152, 2301-04 (1992)). The infection evokes an inflammatory response in the mucosal tissue of the eustachian tube and middle ear, resulting in fluid effusion in the middle ear. The resulting fluid is viscous and pus-filled, making normal mucociliary movement of the fluid difficult, and inflammation of the eustachian tube at its narrowest point, the isthmus, effectively blocks drainage of the fluid into the nasopharnyx (J. Klein, supra (1994)). Middle ear congestion can be expected to cause significant pain, dizziness, and hearing impairment in the patient; the average hearing loss from the fluid accumulation is 25 decibels. This is of particular concern in very young children because impairment of hearing could delay or seriously impede aspects of normal cognitive development which are dependent upon exposure to language and social interaction (D. Teele, et al. *J. Infect Dis* 1621, 685-94 (1990)). Other potential (but uncommon) sequelae of OM include mastoiditis, meningitis, extradural abscess, subdural empyema, brain abscess, and lateral sinus thrombosis.

About 80-90% of OM effusions eventually resolve spontaneously following antibiotic therapy; the process may take as long as three months. However, congestion in the middle ear may persist for weeks or even months beyond sterilization of this fluid with antibiotics due to a continued hypersecretory state of the mucous-producing cells. (S. Wintermeyer and M. Nahata, *Annals of Pharmocotherapy* 28, 1089-99 (1994)). The cause of this persistent hypersecretory state is not well understood but may relate to unrelieved underlying eustacian tube obstruction. As a further impediment to treatment, the effectiveness of antibiotic therapy is decreasing on account of growing bacterial resistance to antibiotics (M. Poole, *Pediatr Infect Dis J.* 14(4), 523-26 (1995)). If middle ear congestion persists for more than three months, surgery is commonly performed to insert a typanostomy tube to ventilate the middle ear of the patient (K. Grundfast, *Arch Otolaryngol Head Neck Surg*, 120, 797-98 (1994)). Tympanostomy surgery is now the second most frequent surgical procedure in children (after circumcision) (J. Klein, supra (1994)). The tube allows drainage of the fluid out of the ear and eventual resolution

of the disease in a vast majority of chronic cases. However, the surgery is costly (> $2,000), and requires administering general anesthesia, a particular concern in infant patients. Furthermore, potential (but uncommon) sequelae of the surgery include persistent otorrhea, permanent perforation or scarring of the tympanic membrane, and cholesteatoma (a cyst-like sac filled with keratin debris that can occlude the middle ear and erode surrounding structures) (J. Klein, supra (1995)).

Thus, as a result of the decreasing effectiveness of antibiotic therapy due to bacterial resistance and the high costs and risks associated with typanostomy surgery, medical researchers have sought to develop other effective therapies for this increasingly prevalent disease. A French biotechnology company, Laboratoires SYNTHELABO FRANCE, has developed a method of treating nasal mucous fluid congestion under the trademark name rhinATP™ which uses adenosine triphosphate (ATP) as the active compound. This technology was licensed under U.S. Patent No. 5,420,116 (applicant intends the disclosure of this and all other patent references and publications cited herein be incorporated herein by reference). Their method of treatment comprises administering ATP to the nasal cavity via nasal spray or nasal drops. Uridine triphosphate (UTP) and adenine triphosphate (ATP) have also been shown to effect the ion transport activity of human airway epithelial cells, as described in U.S. Pat. No. 5,292,498. Specifically, UTP and ATP induce chloride and water secretion by the lung epithelial cells of cystic fibrosis patients, helping to liquify and facilitate transport of the highly viscous airway surface mucus that characterizes this disease. It has also been found that UTP and ATP stimulate the ciliary beat frequency in lung epithelial cells, further facilitating the transport of mucus from the lungs of cystic fibrosis patients. *See*, R. Boucher, et al., Adenosine and Adenine Nucleotides: From Molecular Biology to Integrative Physiology, p. 525-532 entitled "Mechanisms and Therapeutic Actions of Uridine Triphosphates in the Lung" (L. Belardinelli, et al. ed., Alumwer Academic Publishers, Boston 1995); *see also*, L. Gheber, et al., *J. Membrane Biol.* 147, 83-93 (1995). Applicant has discovered that the high viscosity of the retained middle ear fluid in OM patients can be

alleviated by administering UTP and its related compounds, as well as other nucleoside phosphates such as: adenosine 5'-triphosphate (ATP); cytidine 5'-triphosphate (CTP); 1,N^6-ethenoadosine triphosphate; adenosine 1-oxide triphosphate; 3,N^4-ethenocytidine triphosphate; P^1,P^4-di(adenosine-5') tetraphosphate (A_2P_4); or P^1,P^4-di(uridine-5') tetraphosphate (U_2P_4) to the site of fluid blockage.

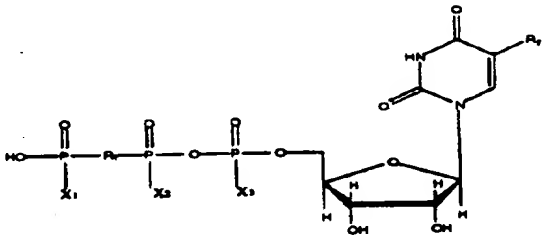
UTP and other nucleoside phosphates induce chloride and water secretion from luminal epithelial cells via activation of the $P2Y_2$ purinergic receptor. The $P2Y_2$ receptor is part of a family of seven transmembrane spanning, G-protein coupled receptors designated the P2Y receptor family. Most members of the P2Y receptor family, including $P2Y_2$, are coupled to the phospholipase C (PLC)-inositol triphosphate (IP_3) pathway (J. Simon, et al., *Eur. J. Pharmacol.*, 291, 281-289 (1995). Recent studies show that agents acting at the $P2Y_2$ purinergic receptor in respiratory epithelia can alter chloride ion transport and other factors which affect mucociliary clearance of mucous secretions (M. Knowles, et al., *New Engl. J. Med.* 325, 533-38 (1991); D. Drutz, et al., *Drug Dev. Res.* 37, 185 (1996)).

Because of UTP's demonstrated ability to enhance clearance of retained mucous secretions via stimulation of the $P2Y_2$ receptor in respiratory epithelium, applicants were motivated to investigate whether the $P2Y_2$ receptor is also expressed in the luminal epithelia of the middle and inner ear.

SUMMARY OF THE INVENTION

A method of treating otitis media in a subject in need of such treatment is disclosed. The method comprises administering to the middle ear of the subject a compound of Formula I, or a pharmaceutically acceptable salt thereof, in an amount effective to promote fluid drainage from the middle ear by hydrating mucous secretions in the middle ear and by increasing ciliary beat frequency in the middle ear and eustachian tube:

Formula I



wherein:

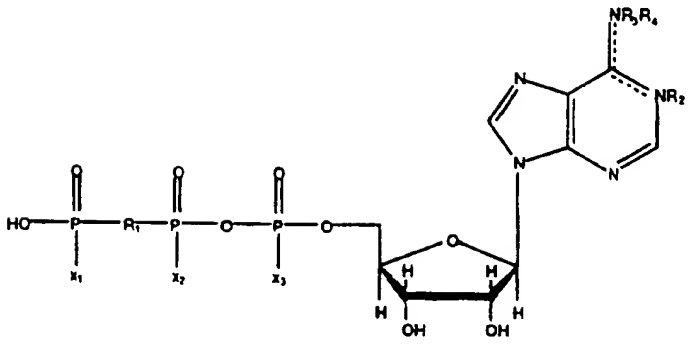
X_1, X_2, and X_3 are each independently either O^- or S^-. Preferably, X_2 and X_3 are O^-.

R_1 is O, imido, methylene, or dihalomethylene (e.g., dichloromethylene, diflouromethylene). Preferably, R_1 is oxygen or difluoromethylene.

R_2 is H or Br. Preferably, R_2 is H. Particularly preferred compounds of Formula I are uridine 5'-triphosphate (UTP) and uridine 5'-O-(3-thiotriphosphate) (UTPγS).

Formula I is the preferred embodiment of the compound, however, the method of the present invention can also include administering a compound of Formula II (adenosine 5' triphosphate [ATP] or $1,N^6$-ethenoadenosine triphosphate or adenosine 1-oxide triphosphate), or Formula III (cytidine 5' triphosphate [CTP] or $3,N^4$-ethenocytidine triphosphate), or Formula IV (P^1,P^4-di(adenosine-5') tetraphosphate (A_2P_4) or P^1,P^4 di(uridine-5') tetraphosphate (U_2P_4).

Formula II



wherein:

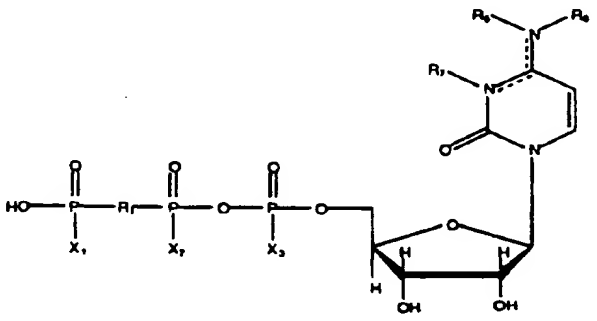
R_1, X_1, X_2, and X_3 are defined as in Formula I.

R_3 and R_4 are H while R_2 is nothing and there is a double bond between N-1 and C-6 (adenine), or

R_3 and R_4 are H while R_2 is O and there is a double bond between N-1 and C-6 (adenine 1-oxide), or

R_3, R_4 and R_2 taken together are -CH=CH-, forming a ring from N-6 to N-1 with a double bond between N-6 and C-6 (1,N^6-ethenoadenine).

Formula III



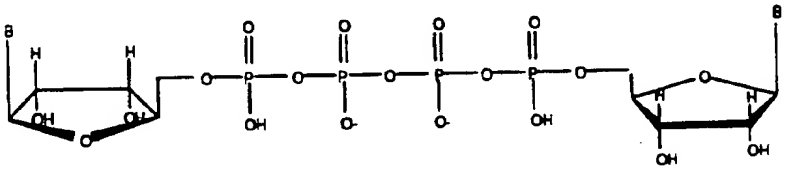
wherein:

R_1, X_1, X_2, and X_3 are defined as in Formula I.

R_5 and R_6 are H while R_7 is nothing and there is a double bond between N-3 and C-4 (cytosine), or,

R_5, R_6 and R_7 taken together are -CH=CH-, forming a ring from N-3 to N-4 with a double bond between N-4 and C-4 (3,N^4-ethenocytosine).

Formula IV



wherein:

B is adenine or uracil.

A second aspect of the present invention is a pharmaceutical formulation containing the compound of Formula I, II, III, or IV in an amount effective to promote fluid drainage from the middle ear by hydrating the mucous secretions in the middle ear and by increasing the ciliary beat frequency in the middle ear and eustachian tube, in a pharmaceutically acceptable carrier.

A third aspect of the present invention is the use of the active compounds disclosed herein for the manufacture of a medicament for the therapeutic hydration of mucous secretions in the middle ear and for the activation of ciliary beat frequency in the middle ear and eustachian tube of apatient in need of such treatment.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

The method of the present invention may be used to promote drainage of congested fluid from the middle and external ear of a subject in need of such treatment for any reason, including (but not limited to) retained secretions arising from middle and external ear diseases such as otitis media, acute otitis media, otitis media with persistent effusion, serous otitis media (arising from an unresolved acute infection, an allergic reaction, or barotrauma such as from rapid descent in an aircraft), or otitis externa. The method of the present invention may also be used to treat inner ear disease, including (but not limited to) Meniere's Disease. The present invention induces drainage of middle ear mucous secretions by hydrating the retained secretions and by increasing the ciliary beat frequency of cilia on the surface of the middle ear and eustachian tube. Hydration of the mucous secretions decreases their viscosity, allowing them to be more easily transported from the middle ear and eustachian tube to the nasopharnyx via mucociliary action. Additionally, the present invention accelerates this mucociliary action, further facilitating drainage of retained middle ear secretions into the nasopharnyx.

The present invention is concerned primarily with the treatment of human subjects, but may also be employed for the treatment of other mammalian subjects, such as dogs and cats, for veterinary purposes.

Compounds illustrative of the compounds of Formula I above include: (a) uridine 5'-triphosphate (UTP); (b) uridine 5'-O-(3-thiotriphosphate) (UTPγS); and (c) 5-bromo-uridine 5'-triphosphate (5-BrUTP). These compounds are known or may be made in accordance with known procedures, or variations thereof which will be apparent to those skilled in the art. See generally N. Cusack and S. Hourani, *Annals N.Y. Acad. Sci.* 603, 172-81 (entitled "Biological Actions of Extracellular ATP"). For example, UTP may be made in the manner described in Kenner, et al., *J. Chem. Soc.* 1954, 2288; or Hall and Khorana, *J. Am. Chem. Soc.* 76, 5056 (1954). See Merck Index, Monograph No. 9795 (11th Ed. 1989). UTPγS may be made in the

manner described in R. S. Goody and F. Eckstein, *J. Am. Chem. Soc.* 93, 6252 (1971).

For simplicity, Formula I herein illustrates uridine triphosphate active compounds in the naturally occuring D configuration, but the present invention also encompasses compounds in the L configuration, and mixtures of compounds in the D and L configurations, unless otherwise specified. The naturally occuring D configuration is preferred.

Compounds illustrative of the compounds of Formula II above include (a) adenosine 5'-triphosphate (ATP) and (b) $1,N^6$-ethenoadenosine triphosphate. Compounds illustrative of the compounds of Formula III above include (a) cytidine 5'-triphosphate and (b) $3,N^4$-ethenocytidine triphosphate. These compounds can be made in accordance with known procedures, or variations thereof which will be apparent to those skilled in the art. For example, phosphorylation of nucleosides by standard methods such as D. Hoard and D. Ott, *J. Am. Chem. Soc.* 87, 1785-1788 (1965); M. Yoshikawa, et al., *Tetrahedron Lett.* 5065-68 (1967) and *idem., Bull. Chem. Soc. (Jpn)* 42, 3505-08 (1969); J. Moffatt and H. Khorana, *J. Am. Chem. Soc.* 83, 649-59 (1961); and B. Fischer, et al., *J. Med. Chem.* 36, 3937-46 (1993) and references therein. Etheno derivatives of cytidine and adenosine are prepared by known methods such as: N. Kotchetkov, et al., *Tetrahedron Lett.* 1993 (1971); J. Barrio, et al., *Biochem. Biophys. Res. Commun.* 46, 597 (1972); J. Secrist, et al., *Biochemistry* 11, 3499 (1972); J. Bierndt, et al., *Nucleic Acids Res.* 5, 789 (1978); K. Koyasuga-Mikado, et al., *Chem. Pharm. Bull. (Tokyo)* 28, 932 (1980). Derivatives with alpha, beta and gamma thiophosphorus groups can be derived by the following or by adapting methods of: J. Ludwig and F. Eckstein, *J. Org. Chem.* 54, 631-35 (1989); F. Eckstein and R. Goody, *Biochemistry* 15, 1685 (1976); R. Goody and F. Eckstein, *J. Am. Chem. Soc.* 93, 6252 (1971).

Compounds of Formulas I, II, or III where R_1 is CCl_2 and CF_2 can be prepared by methods similar to that described in G. Blackburn, et al., *J. Chem. Soc. Perkin Trans.* I, 1119-25 (1984). Compounds of Formula I, II, III where R_1 is CH_2 can be prepared by methods similar to that described in T. Myers, et al., *J. Am. Chem. Soc.* 85, 3292-95 (1963).

Compounds illustrative of the compounds of Formula IV include (P^1,P^4-di(adenosine-5') tetraphosphate (A_2P_4) or P^1,P^4-di(uridine-5') tetraphosphate (U_2P_4). These compounds can be made in accordance with known procedures, or variations thereof which will be described by: P. Zamecnik, et al., *Proc. Natl. Acad. Sci. USA* 89, 838-42 (1981); and K. Ng and L. E. Orgel, *Nucleic Acids Res.* 15 (8), 3572-80 (1987).

In addition, UTP, ATP, CTP, A_2P_4, 3,N^4-ethenocytidine triphosphate, 1,N^6-ethenoadenine triphosphate, adenosine 1-oxide triphosphate, ATPγS, ATPβS, ATPαS, AMPPCH$_2$P, AMPPNHP, N^4-ethenocytidine and 1,N^6-ethenoadenosine are commercially available, for example, from Sigma Chemical Company, PO Box 14508, St. Louis, MO 63178.

The active compounds of Formulae I - IV may be administered by themselves or in the form of their pharmaceutically acceptable salts, e.g., an alkali metal salt such as sodium or potassium, an alkaline earth metal salts such as manganese, magnesium and calcium or an ammonium and tetraalkyl ammonium salts, NX_4^+ (wherein X is C_{1-4}). Pharmaceutically acceptable salts are salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects.

The active compounds disclosed herein may be administered to the middle ear of a patient to promote fluid drainage in otitis media by a variety of suitable means, but are preferably administered by administering a liquid/liquid suspension (either a nasal spray of respirable particles which the subject inhales, or nasal drops of a liquid formulation) comprised of the active compound. Liquid pharmaceutical compositions of the active compound for producing a nasal spray or nasal drops may be prepared by combining the active compound with a suitable vehicle, such as sterile pyrogen free water or sterile saline by techniques known to those skilled in the art.

The dosage of active compound to promote fluid drainage will vary depending on the condition being treated and the state of the subject, but generally an effective amount is the amount sufficient to achieve concentrations of active compound on the middle ear surfaces

of the subject of from about 10^{-7} to about 10^{-2} Moles/liter, and more preferable from about 10^{-6} to about 3×10^{-4} Moles/liter.

Depending upon the solubility of the particular formulation of active compound administered, the daily dose to promote fluid drainage may be divided among one or several unit dose administrations. Preferably, the daily dose is no more than two times per day.

Another means of administering the active compound to the middle ear of the patient to promote fluid drainage may include any oral form of the active compound, administered to the patient either by means of a liquid suspension of the active compound which is poured into the mouth of the patient, or by means of a pill form swallowed by the patient.

Another means of administering an effective amount of the active compound to the middle and inner ear would involve the patient inhaling a nebulized form of the active compound into their respiratory tract, such that the active compound enters the nasopharnyx and subsequently enters the inner and middle ear of the patient.

Another means of administering the active compound to the middle ear would include any topical form of the active compound, administered as a cream or gel to the outer ear, which would subsequently permeate through the tympanic membrane into the middle ear of the patient.

Another means of administering the active compound to the middle ear would involve an injected form of the active compound, injected from the outer ear directly through the tympanic membrane into the middle ear, or injected indirectly through the upper neck region into the middle ear.

Another means of administering the active compound to the middle ear would involve a suppository form of the active compound, such that a therapeutically effective amount of the compound reaches the middle ear via systemic absorption.

An additional means of administering the active compound would involve intra-operative instillation of the active compound into the middle, inner or outer ear via a gel, cream, or

liquid suspension form of the active compound, such that a therapeutically effective amount reaches the middle, inner or outer ear.

UTP and compounds of Formulae I - IV also have therapeutic benefit when used in combination with other agents used to treat otitis media, such as, but not limited to: antibiotics like penicillin, penicillan plus beta-lactam, erythromycin plus sulisoxazole, ephalosporin, trimethodprim, trimethodprim plus sulfamethoxazole, macrolides, and oxazolidinones; vaccines; antihistamines, decongestants, mucolytic agents; nonsteroidal antiinflammatory agents; and corticosteroids. UTP may also be used in combination with agents under development, such as NE-1530--a naturally occuring airway oligosaccharide (Neose Technologies, Inc.), and gene therapy.

The present invention is explained in greater detail in the Examples which follow. These examples are intended as illustrative of the invention, and are not to be taken as limiting thereof.

EXPERIMENTAL

Example 1

$P2Y_2$ Expression in the Human Eustachian Tube

In situ analysis of the mRNA for the $P2Y_2$ receptor was performed on sections of human Eustachian tube epithelia. This procedure was adapted from L. Burch, et al., *Am. J. Physiol.*, 269(2), C511-C518 (1995). Frozen sections (8 μm) were mounted on slides and fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 2 h. After fixation, slides were rinsed twice in PBS, dehydrated, air dried, and stored at -80°C until use. Prehybridization treatments consisted of proteinase K digestion then acetylation. RNase control sections were treated with 200 mg/ml RNase A. Slides containing serial sections were hybridized overnight at 54°C in a hybridization buffer containing 10^6 counts/min of either antisense or sense probes. ^{35}S-UTP-labeled RNA probes were synthesized with the Ambion MAXIscript in vitro transcription system. After hybridization, slides were washed in 4 x SSC at room temperature, followed by RNase A digestion (20 mg/ml), then 2 x SSC/1 mM dithiothreitol (DTT) at room temperature and a high-stringency wash of 0.5 x SSC/1 mM DTT at 54°C (3 x 15 min), followed by ethanol dehydration. Dried slides were dipped in Kodak NTB2 photoemulsion diluted 1:1 with 0.6 M ammonium acetate. Slides were developed at intervals from 1.5 to 2.5 wk, and counterstained with hematoxylin and eosin.

Fig. 1

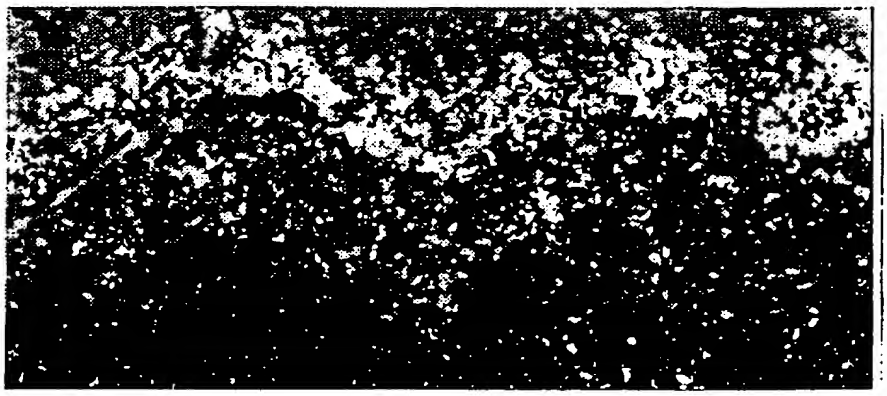


Fig. 2

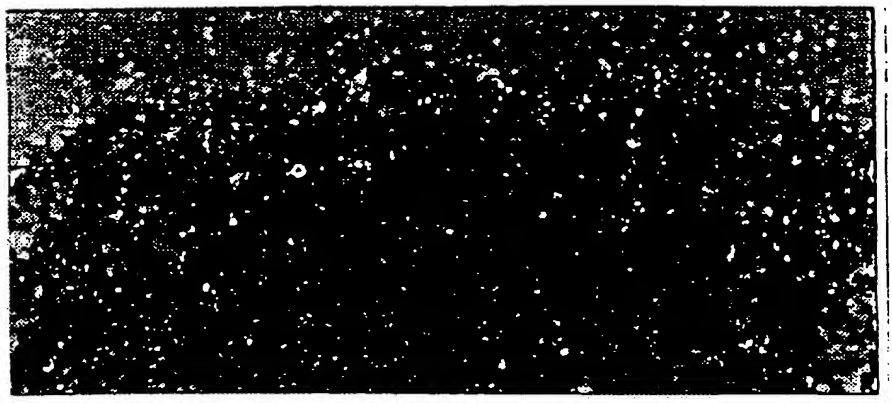


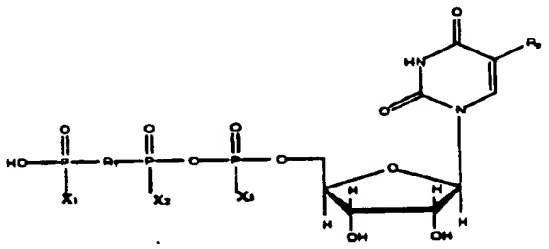
Figure 1 is the in situ hybridization of human $P2Y_2$ receptor using a 600 bp antisense probe. Figure 2 is the in situ hybridization of human $P2Y_2$ using, as a control, a 600 bp sense probe. Comparison of antisense and sense probes reveals more radioautographic signal with antisense probe, consistent with expression of $P2Y_2$ receptor in the Eustachian tube.

WHAT IS CLAIMED IS:

1. A method of treating otitis media in a subject in need of such treatment, said method comprising:

administering to the middle ear of the subject a compound of Formula I, II, III, or IV, or a pharmaceutically acceptable salt thereof, in a pharmaceutical carrier having an amount of said compound effective to promote fluid drainage from the middle ear:

Formula I



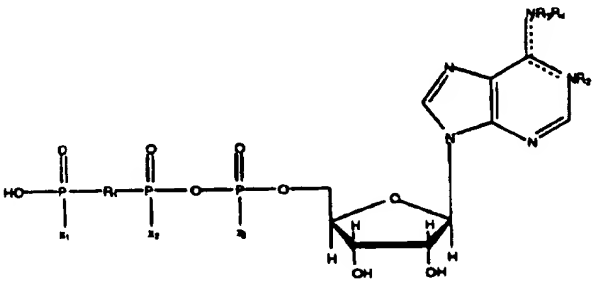
wherein:

X_1, X_2, and X_3 are each independently selected from the group consisting of OH and SH:

R_1 is selected from the group consisting of O, imido, methylene, and dihalomethylene; and

R_2 is selected from the group consisting of H and Br

Formula II



wherein:

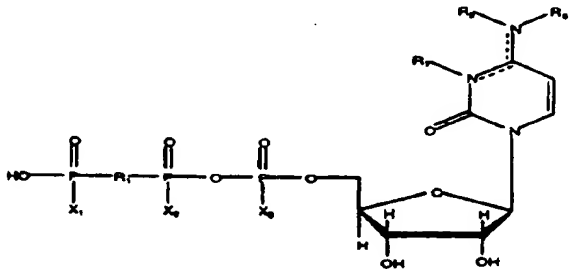
R_1, X_1, X_2, and X_3 are defined as in Formula I.

R_3 and R_4 are H while R_2 is nothing and there is a double bond between N-1 and C-6 (adenine), or

R_3 and R_4 are H while R_2 is O and there is a double bond between N-1 and C-6 (adenine 1-oxide), or

R_3, R_4 and R_2 taken together are -CH=CH-, forming a ring from N-6 to N-1 with a double bond between N-6 and C-6 (1,N^6-ethenoadenine)

Formula III



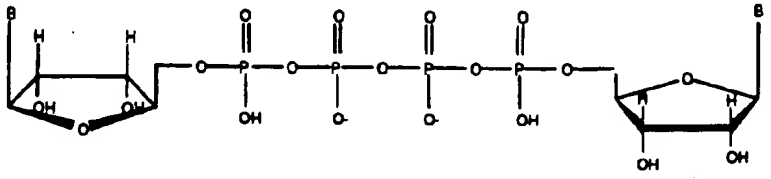
wherein:

R_1, X_1, X_2, and X_3 are defined as in Formula I.

R_5 and R_6 are H while R_7 is nothing and there is a double bond between N-3 and C-4 (cytosine), or,

R_5, R_6 and R_7 taken together are -CH=CH-, forming a ring from N-3 to N-4 with a double bond between N-4 and C-4 (3,N^4-ethenocytosine)

Formula IV



wherein:

B is adenine or uracil.

2. A method according to Claim 1, wherein said compound is delivered by administering a liquid/liquid suspension, including nasal drops or spray, of said compound to the nasopharngeal airways of said subject, such that a therapeutically effective amount of said compound contacts the eustachian tube or middle ear of said subject either directly or via systemic absorption and circulation.

3. A method according to Claim 1, wherein said compound is delivered by administering an oral form of said compound to the middle ear of said subject, such that a therapeutically effective amount of said compound contacts the eustachian tube or middle ear of said subject via systemic absorption and circulation.

4. A method according to Claim 1, wherein said compound is delivered by administering an aerosol suspension of said compound to the nasopharyngeal airways of said subject, such that a therapeutically effective amount of said compound contacts the eustachian tube or middle ear of said subject.

5. A method according to Claim 1, wherein said compound is delivered by administering a topical form of said compound to the middle ear, via the tympanic membrane of said subject, such that a therapeutically effective amount of said compound contacts the eustachian tube or middle ear of said subject.

6. A method according to Claim 1, wherein said compound is delivered by administering an injected form of said compound, such that a therapeutically effective amount of said compound contacts the eustachian tube or middle ear of said subject.

7. A method according to Claim 1, wherein said compound is delivered by administering a suppository form of said compound, such that a therapeutically effective amount of said compound contacts the eustachian tube or middle ear of said subject via systemic absorption and circulation.

8. A method according to Claim 1, wherein said compound is administered in an amount sufficient to achieve concentrations thereof on the middle ear or eustachian tube surfaces of said subject of from about 10^{-7} to about 10^{-2} Moles/liter.

9. A method according to Claim 1, wherein X_2 and X_3 are OH.

10. A method according to Claim 1, wherein R_1 is oxygen.

11. A method according to Claim 1, wherein R_2 is H.

12. A method according to Claim 1, wherein said compound of Formula I is selected from the group consisting of uridine 5'-triphosphate, uridine 5'-O-(3-thiotriphosphate), 5-bromo-uridine 5'-triphosphate and the pharmaceutically acceptable salts thereof.

13. A method according to Claim 1, wherein said compound of Formula II is selected from the group consisting of adenosine 5'-triphosphate, $1,N^6$-ethenoadenosine triphosphate, adenosine 1-oxide triphosphate and the pharmaceutically acceptable salts thereof.

14. A method according to Claim 1, wherein said compound of Formula III is selected from the group consisting of cytidine 5'-triphosphate (CTP), $3,N^4$-ethenocytidine triphosphate and the pharmaceutically acceptable salts thereof.

15. A method according to Claim 1, wherein said compound of Formula IV is selected from the group consisting of P^1,P^4-

di(adenosine-5′) tetraphosphate (A_2P_4) and P^1,P^4-di(uridine-5′) tetraphosphate (U_2P_4) and the pharmaceutically acceptable salts thereof.

INTERNATIONAL SEARCH REPORT

Interna..al Application No
PCT/US 97/02299

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 292 498 A (BOUCHER JR RICHARD C) 8 March 1994 see claim 11 ---	1-15
Y	INT. J. OF PEDIATRIC OTORHINOL., vol. 10, 1985, pages 47-52, XP000654070 J. NUUTINEN: "Activation of the impaired nasal mucociliary...." * summary; p.47. 1st par. * ---	1-15
Y	THE AMERICAN J. OF OTOLOGY, vol. 9, no. 5, 1988, pages 418-22, XP000654235 MIZUKOSHI ET AL: "Clinical evaluation of medical treatment for meniere's..." see the whole document --- -/--	1-15

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
5 June 1997	20.06.97

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

Interna..al Application No
PCT/US 97/02299

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CLINICAL PEDIATRICS, vol. 34, 1995, pages 542-48, XP000654269 T. E. LISTON: "Management of Otitis media" see the whole document ---	1-15
P,X	SCRIP, vol. 2151, August 1996, page 14 XP002032361 "US Orphan status for Inspire's UTP" * left hand col., last par. * -----	1-15

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Interna al Application No
PCT/US 97/02299

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5292498 A	08-03-94	WO 9408593 A	28-04-94
		AU 2872592 A	09-05-94
		EP 0663830 A	26-07-95
		JP 8502078 T	05-03-96
		NO 951340 A	02-06-95